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Supercritical fluid extraction of *Vernonia galamensis* seeds

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Abstract

Vernonia galamensis is an excellent source of a seed oil rich in vernolic acid. However, the seeds of this plant exhibit a high lipase activity in the dormant state. The activity of this enzyme is apparent as vernonia oil will undergo lipolysis when the seeds are crushed prior to extraction. In this study, supercritical fluid extraction (SFE) utilizing carbon dioxide has been examined as an alternative solvent, at different pressures, temperatures and cosolvent concentrations. The use of supercritical fluid carbon dioxide (SC-CO_2) as the extraction fluid under high temperatures and pressures has the potential to inhibit the lipolysis reaction during the extraction by reducing the activity of the enzyme, and hence the production of undesired fatty acids. Vernonia seeds were ground with dry ice prior to extraction to minimize any nascent lipase activity. Selective SFE was conducted at various pressures, temperatures, modifier concentrations, and total CO_2 volume used to determine whether the vernolic acid content of the resultant extract could be enriched. An increase in pressure and temperature significantly increased the amount of extracted oil as well as the vernolic acid content. In addition, a significant reduction in the free fatty acid content of the oil from 69 to 8 mg/g oil was present with increasing extraction pressure. Exhaustive extraction of the oil could be attained via SFE with neat CO_2 and with ethanol-modified CO_2. However, regrinding the matrix after the initial extraction, followed by re-extraction of the seed matrix was necessary in these cases. The resultant oil and extracted meal were characterized with respect to free fatty acid, phospholipid, and percent protein contents. Published by Elsevier Science B.V.

Keywords: Extraction; Lipase; Supercritical fluid; Vernonia

1. Introduction

Vernonia galamensis seeds are characterized by the rapid induction of lipolytic activity when the seeds are crushed, leading to high free fatty acid levels in the extracted oil and resultant meal. Therefore, proper precautions should be taken by introducing a heating step in the oil extraction processing scheme (Carlson et al. 1981; Ayorinde et al. 1990; Mohamed et al., 1995). Toward this end, Mohamed et al. (1995) have shown that

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microwave heating may be even more efficient than conventional heating for deactivating the lipase. Aside from the application of heat, lipase activity may also be minimized by pressure-induced denaturation, which is the basis of a new technology known as high pressure food processing (Issacs, 1998).

Supercritical fluid extraction (SFE) is an alternative extraction method whereby supercritical fluids, instead of organic solvents, are used as an extraction medium. Extraction with supercritical carbon dioxide (SC-CO_2) can offer advantages when processing seeds for their oil and meal content (King and List, 1996). Among these benefits are elimination of solvent residues in the extracted oil and meal, environmental compatibility, and end-products having superior properties to conventionally-extracted oils (List et al., 1989). It is also possible to selectively fractionate seed oils by changing the density of the SC-CO_2, thereby producing an oil with desirable physical properties and/or chemical composition (Favati et al., 1991). Additional benefits may accrue from supercritical fluid processing with respect to meal properties, among which are improved color and odor, functionality, and elimination of residual enzymatic activity (Favati et al., 1996).

Vernonia seeds contain approximately 40% oil, composed of a valuable and functional epoxy-containing fatty acid or corresponding triglyceride, known as vernolic acid (*cis*-12,13-epoxy-*cis*-9-octadecanoic acid). Vernonia meal has potential as an alternative protein source (> 40%). It also contains an active lipase, which has unique acidolysis and hydrolysis selectivity properties toward vernolic acid (Ayorinde et al., 1993; Aldercreutz et al., 1997). Vernonia oil has been characterized by several investigators (Neff et al., 1993; Anderson et al., 1993) with respect to its molecular composition and shown to contain up to 80% vernolic acid. Recently, analytical supercritical fluid chromatography (SFC) has been applied for the analysis of vernolic acid in *Euphorbia lagascae* oil by Borch-Jensen and Mollerup (1996), thus demonstrating at least a nascent solubility of the oxy-functional fatty acid in SC-CO_2.

In this study, SFE was applied to extract oil from vernonia seeds, which were obtained from Ver-Tech, Inc. (Plano, TX). The optimal conditions for efficient extraction were determined and the results of this method of extraction on oil and meal characteristics determined. SFE of oils from their native seed matrices is impacted by a number of factors other than just the solubility of the oil in neat SC-CO_2 (King, 1997). Of particular interest was the effect of extraction with SC-CO_2 on the production of residual free fatty acids in the extracted oil and seed meal. SFEs were conducted at various pressures, temperatures, and quantity of CO_2 used; with and without the addition of a GRAS (Generally Regarded as Safe) cosolvent, ethanol, to observe the effect of fluid density on the composition and properties of the vernonia oil and meal. The effects of seed pretreatment and grinding were also noted.

2. Materials and methods

2.1. Materials

Cleaned V029-vernonia seeds were utilized in the two SF extraction techniques employed for oil extraction. For all the extractions, the seeds were mixed with dry ice and ground in a Model MC-170 Miracle Mill (Markson Science Inc., Phoenix, AZ) to a fine powder to minimize initial lipolysis.

2.2. Methods

Initial experiments on ground seeds were performed on 5 g samples of vernonia seeds using an Applied Separations Spe-ed extractor (Allentown, PA). These experiments were used to determine the optimum pressure and temperature for extraction, while holding the volume of CO_2 used constant. In these experiments, the volume of CO_2 used did not exhaustively extract the matrix, but it allowed the effect of pressure and temperature on the amount of vernonia oil extracted to be ascertained. These extractions were performed at 13.8, 34.5 and 69 MPa and at 40°, 60°, 80° and 100°C, employing 100 l of neat CO_2 (measured at ambient conditions). The extracted oil was collected after depressurization in 20 ml vials.

Additional experiments were performed on an Isco SFX 2-10 extraction system (Isco Inc., Lincoln, NE) utilizing a cosolvent pump. This is an analytical-scale extractor using smaller extraction vessels (10 ml). Thus, the amount of seed was reduced to approximately 2 g for these extractions. Two sets of pressure/temperature parameters (51.7 MPa/80°C & 69 MPa/100°C) were used while adding 5, 10 and 15% (v/v) ethanol to the CO_2. Again, the volume of fluid used did not exhaustively extract the matrix, but it allowed the effects of a cosolvent (ethanol) on oil extraction to be observed.

In contrast to the above experiments, which were partially designed to conserve seed matrix use, SFE experiments were also executed with laboratory-constructed extractors to obtain complete extraction of the vernonia oil. This extraction equipment and procedure have been described in the literature (Favati et al., 1991). Vernonia seeds contain between 36 and 43% oil, and to achieve complete extraction, the seed matrix was removed from the extraction cell (15.5 cm long × 1.6 cm i.d.) after performing an initial extraction and reground. The second SFE was then performed on the finer comminuted vernonia, allowing exhaustive extraction of the vernonia oil to be accomplished. The double-step SFE procedure was performed on the vernonia seed (5 g) at 69 MPa, 100°C, using 600 l CO_2 (the seed was reground after 300 l) and an expanded CO_2 flow rate of approximately 4.0 l/min. The dual SFE procedure was also done using the Isco system, using 2 g of vernonia seed, at 69 MPa, 100°C and 400 ml of 5% EtOH/CO_2 as the extraction fluid. In this case, the sample was reground after 200 ml.

2.3. Analysis and characterization of extracted oil and meal

2.3.1. Determination of oil content

The oil extracted was done by differential gravimetry on the oil sample collected after decompression. Dry vials were tared before commencing the SFE in the case of the experiments conducted on the Spe-ed and Isco extractor units. For extractions where the laboratory-constructed SFE units were used, the connecting tubing and adapters were rinsed with 5 ml quantities of hexane, acetone, and then methylene chloride, consecutively, after the conclusion of the extraction. The rinse solution was then concentrated by removing the volatile solvents with heat and nitrogen sparge before weighing the oil.

2.3.2. Determination of vernolic acid content of vernonia oil

Fatty acid methyl esters (FAME) were prepared by incubation of 1 mg oil with 0.5 ml of boron trifluoride in methanol (14%, w/v) at 65°C for 30 min. After cooling to room temperature, 1 ml of hexane followed by 1 ml of water saturated with NaCl were added. The mixture was shaken and then centrifuged at 3000 × g for 3 min and the hexane layer containing the FAME was removed, dried over anhydrous Na_2SO_4, and analyzed by gas chromatography (GC). For the GC analysis, a Supelco Wax 10 capillary column (Supelco Inc., Bellefonte, PA, 30 m × 0.25 mm i.d., 0.25 film thickness and a Hewlett-Packard Model 5890A GC (Hewlett-Packard, Wilmington, DE) equipped with a flame ionization detector (FID) and mass spectrometer detector (MSD) was employed. Helium was used as the carrier gas at a flow rate of 52.5 ml/ min with split ratio of 100:1. The oven temperature was held isothermally at 200°C. The injector and detector temperatures were set at 250° and 280°C, respectively. To analyze for vernolic acid, the chromatographic run time was extended to 25 min. FAMEs were identified by comparison with standard FAME mixture. Vernolic acid was purified from 20 g of vernonia oil and used as a standard. Quantification was made using heptadecanoic acid (17:0) as an internal standard. The vernolic acid content was calculated as relative weight percentage of total fatty acids, using a normalization technique to calculate absolute response factors for vernolic acid. The reported values are averages of three separate determinations.

2.3.3. Determination of free fatty acids

Free fatty acids were determined using the method described by Nixon and Chan (1979). In this method, 5 mg meal was extracted using 10 ml

of a chloroform: *n*-heptane: methanol (4:3:2, v/v/v) mixture. After centrifugation the supernatant was washed, 2 ml of copper reagent were added to 5 ml of chloroform/heptane method extract. The tubes containing the above solution were then vortexed for 3 min, then centrifuged for 5 min at 3000 rpm. Three milliliters of the organic supernatant was then transferred into a clean test tube. A 0.2 ml aliquot of color reagent was then added to each tube, mixed carefully, and the resultant color allowed to develop for 20 min at room temperature. The solution absorbance was read at 550 nm against a reagent blank consisting of 3 ml (chloroform: *n*-heptane: methanol at 4:3:2 v/v). Linolenic acid standards ranging from 10–130 nmol were used to establish a standard curve. Free fatty acid content was calculated as mg/g of meal.

2.3.4. Determination of phospholipids

Total phospholipids were determined in vernonia seed oil as described by Mohamed et al. (1995). Approximately 0.1 ml of the extracted oil was mixed with 0.2 ml of a chromogenic solution and the mixture was heated at 90°C for 10 min. The tubes were then cooled to room temperature and 5 ml chloroform were added. The chloroform layer was carefully removed and the developed color was read at 710 nm against a blank solution. Phosphatidylcholine standards ranging from 50 to 1000 μg per tube were used to establish a standard curve.

2.3.5. Determination of total protein

Protein content was analyzed by the Kjeldahl method. After complete digestion, N_2 was determined using Nessler reagent. To calculate % protein, the N_2 value was multiplied 6.25.

3. Results and discussion

3.1. Extraction of oil

The total percent oil extracted in the limited volume CO_2 experiments as a function of pressure and temperature are shown in Fig. 1. At 13.8 MPa, a maximum amount of oil was extracted at the lower temperatures, a reflection of the density increase of the extraction fluid (SC-CO_2) at this degree of compression. At 34.5 MPa, the oil solubility was 7.9 wt%, increasing to 10.9 and 11.4 wt% at 60° and 80°C, respectively, before decreasing to 7.2 wt% at 100°C. This trend is partially reflective of the "crossover effect" (King, 1997) where a density-based reduction in oil solubility with temperature is counteracted by an increase in oil solubilization in the supercritical fluid due to the increase in the vernonia oil's vapor pressure with increasing temperature. At 69 MPa, oil solubility increased with temperature as noted by Freidrich (1984) and other investigators. Clearly, for exhaustive oil extraction, a combination of 69 MPa and 100°C is preferred.

Under similar extraction conditions to those reported in Fig. 1, the vernolic acid content is shown in Fig. 2. At 13.8 MPa, the vernolic acid content was invariant with respect to temperature, however for extractions conducted at 34.5 MPa, a steady increase in the vernolic acid content of the extracted oil with temperature occurred, ranging from 49.4% at 40°C to 63.4% at 100°C. This suggests conditions for fractionating this particular vernonia oil into fractions enriched in vernolic acid, as noted by Carlson et al. (1981), for the hexane extraction of vernonia oil. At 69 MPa as a function of temperature, there is no noticeable

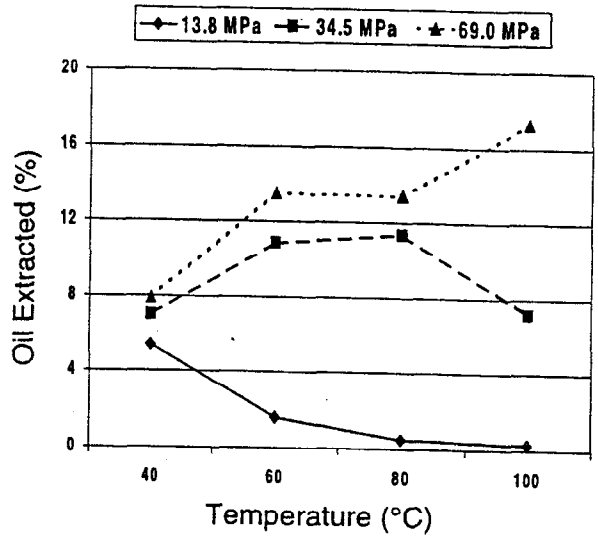

Fig. 1. Effect of temperature and pressure on oil extraction from vernonia seeds.

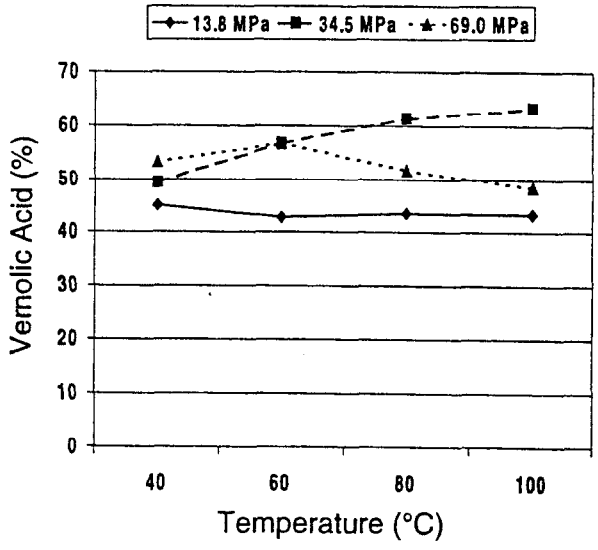

Fig. 2. Effect of temperature and pressure on the vernolic acid content of extracted vernonia oil.

trend in vernolic acid content of the oil extract. However the vernolic acid (ranging from 48.5 to 56.8%) is similar to results recorded at 34.5 MPa extraction pressure and higher than those found in the 13.8 MPa extracts.

The free fatty acid content of the extracted oil as a function of temperature and pressure are shown in Fig. 3. At 13.8 MPa, a definite reduction in the free fatty acid content occurred as the extraction temperature increased from 40° to 100°C, decreasing from 69.3 to 12 mg/g-oil, or 6.9 to 1.2 wt%. At 34.5 MPa, there is little change in the free fatty acid content of the oil with extraction temperature. However a substantial decrease in free fatty acid content was observed when the extraction was done at 40°C when increasing the extraction pressure from 13.8 to 34.5 MPa. The values of free fatty acid content of the oil are recorded at 69 MPa extraction pressure, ranging from 1.89 to 0.84 wt%. These are similar values to those recorded by Carlson et al. (1981) with hexane extractions on vernonia oil. These results also suggest that the extraction pressure as well as the extraction temperature can be used to minimize the production of free fatty acids due to lypolysis, i.e. that extractions conducted at 69 MPa and 100°C are most favorable to minimizing free fatty acid formation.

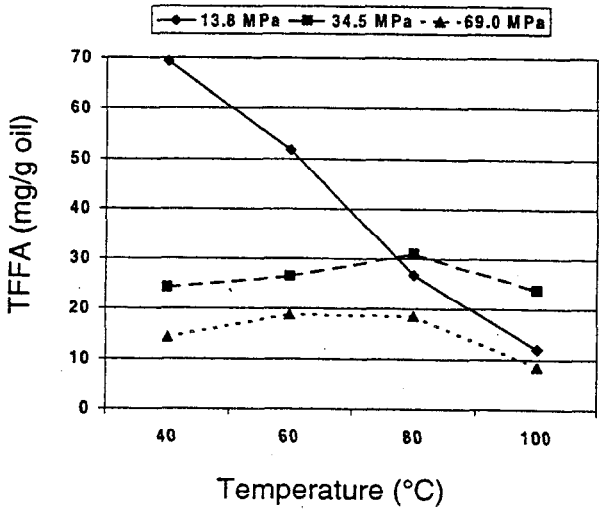

Fig. 3. Effect of temperature and pressure on the total free fatty acid (TFFA) content of extracted vernonia oil.

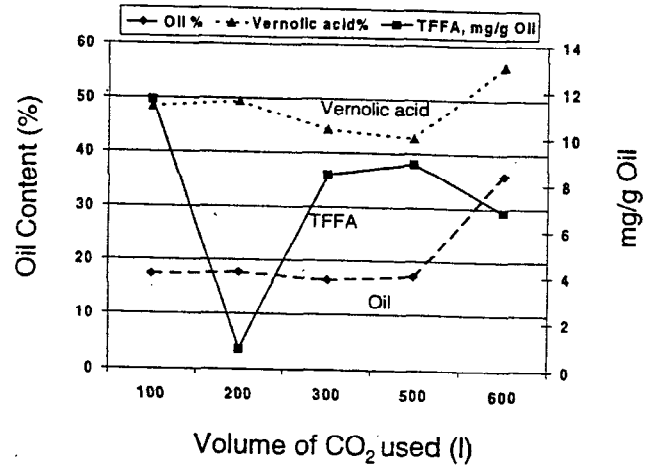

Fig. 4. Effect of CO_2 volume on oil extraction, vernolic acid content, and total free fatty acid content at 69 MPa and 100°C.

The effect of CO_2 processing volume is shown in Fig. 4 where the oil, vernolic acid, and total free fatty acids (TFFA) contents are related to the liters CO_2 passed through the extraction cell in 100 l intervals for a vernonia oil extraction conducted at 69 MPa and 100°C. Increasing the

volume of CO_2 used in the extraction from 100 to 500 l, did not increase the overall extractability of oil. Increasing the volume of CO_2 to 600 l however completed the oil extraction (36%). Similarly, at 600 l of CO_2 passage, the greatest amount of vernolic acid (56.2%) was achieved. With the exception of an anomalously low total free fatty acid value after 200 l of CO_2 passage, the TFFA values decreased with passage of CO_2 from 1.16 to 0.685 wt%. As noted in the materials and methods section, a double SFE extraction was carried out with ground seed initially extracted by 300 l of CO_2, then the meal was removed, reground, and reextracted again with another 300 l of CO_2. This double SFE extraction procedure also proved efficient in yielding a complete extraction of the oil. Similar results were also attained when 5% ethanol was used as a cosolvent at 69 MPa and 100°C, and passage of 400 ml of liquid CO_2.

To better study the effect of cosolvent addition, ethanol was added to the extraction at 5, 10, and 15 vol% levels. Two conditions of pressure and temperature were selected (51.7 MPa/80°C and 69 MPa/100°C) for extracting the vernonia oil with the aid of the cosolvent. As shown in Fig. 5, the percent of extracted mass increased with ethanol addition significantly, both at 51.7 MPa/80°C and 69 MPa/100°C. At both combinations of pressure and temperature, a 15 vol% addition of ethanol resulted in over a 45% mass recovery (Fig. 5). This extract probably contains other moieties besides vernonia oil, such as polar lipids, water, and perhaps some protein and carbohydrate (Montanari et al., 1999). These extractions also showed ethanol addition reduced the CO_2 volume needed for full extraction of the oil.

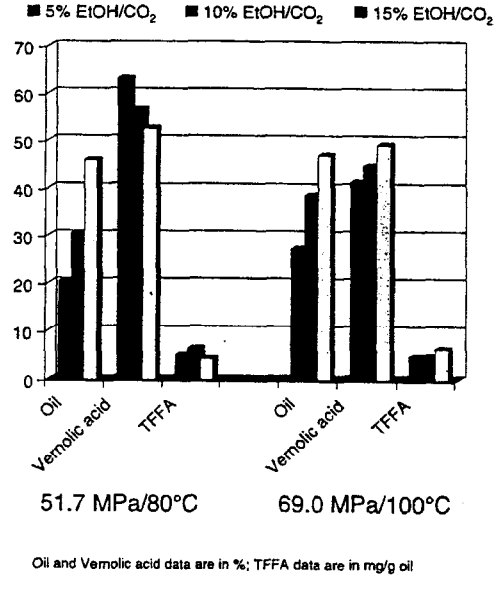

Fig. 5. Vernonia oil yield, vernolic acid content, and total free fatty acid content as a function of pressure, temperature, and level of cosolvent addition.

Table 1
Effect of pressure and temperature on the fatty acid content of extracted vernonia seed meal after SFE

Total free fatty acid (mg-acid/g-meal)			
	Pressure		
Temperature (°C)	13. 8 Mpa	34.5 Mpa	69 MPa
40	0.608	0.583	0.405
60	0.455	–	–
80	0.416	0.379	0.323
100	0.316	0.324	0.307

With respect to the vernolic acid content, extraction at 51.7 MPa/80°C, at 5 vol% addition of ethanol maximized the vernolic content of the oil (Fig. 5). The vernolic acid content is reduced under these same conditions when the ethanol content of the extraction fluid is increased to 15 vol% (from 63 to 53%). However the inverse trend is observed at 69 MPa/ 100°C where the vernolic acid content increased from 41 to 49% as the ethanol volume percentage in SC-CO_2 increased from 5 to 15 vol%. These results suggest some interesting possibilities for regulating the vernolic acid content of the final oil extract.

The TFFA content of the oil extractions performed with the addition of ethanol as a cosolvent is also summarized in Fig. 5. No discernible trend in the TFFA content with in-

creasing ethanol content was observed at either set of extraction conditions. The TFFA values are very low (0.41–0.63 wt%) for the extraction conditions studied.

3.2. Characterization of extracted vernonia meals

Although the quantity of extracted meal was limited for some of the reported extractions, TFFA, protein content, and phospholipid level were determined whenever possible on the extracted vernonia meals. As summarized in Table 1, there is a correlation between the TFFA content of the extracted meal and the extraction pressures and temperatures that have been applied. A multiple regression yielded the following relationship between TFFA content and the extraction temperature (T) and pressure (P) as given in Eq. (1):

$$1/\text{TFFA content} = 0.937 + 0.02*T + 5.255 \times 10^{-13} \times P^3 \quad (1)$$

having an R^2 value of 0.94. Using the above equation, it was shown that the numbers reported in Table 1 are statistically different and support the following conclusions.

At 13.8 MPa, the application of increasing temperature significantly lowers the TFFA content of the meal from 0.608 to 0.316 mg/g-meal. These temperature-dependent significant differences also persist with increasing pressure as indicated by the results at 34.5 and 69 MPa (Table 1). At any given temperature in Table 1, there is also a gradual lowering of the TFFA content of the meal as pressure is increased.

Table 2
Effect of carbon dioxide volume on the fatty acid content of extracted vernonia meal at 100°C and 69 MPa

Volume of CO_2 (l)	Total free fatty acids (mg-acid/g-meal)
100	0.307
300	0.286
500	0.224
600	0.176

This is consistent with a pressure-based deactivation of the lipase and is identical to the trends reported in Fig. 3 for TFFA levels found in the extracted oil under similar conditions.

Extraction time (passage of CO_2) also aids in deactivating enzymatic activity in the vernonia meal. As shown in Table 2, the TFFA level drops from a value of 0.307 mg/g-meal after 100 l-CO_2 exposure to 0.176 mg/g-meal after passage of 600 l of expanded CO_2 for extractions conducted at 69 MPa and 100°C. The regression Eq. (2) for the relationship between the volume of CO_2 and TFFA content was found to be:

$$\text{TFFA content} = 0.315 - 3.782 \times 10^{-7}*(\text{CO}_2 \text{ volume})^2 \quad (2)$$

yielding an R^2 value of 0.99. Predicted TFFA values for the passage of 100 and 300 l of CO_2 were found not be statistically different based on the overlap of the regression confidance intervals at the 95% level. This conclusion also held true for the predicted TFFA values for 500 and 600 l of CO_2, however the TFFA values at 100 and 300 l of CO_2 are statistically different from those at 500 and 600 l of CO_2 supporting the conclusion that the TFFA content decreases with passage of CO_2.

The protein content in the extracted meals as a function of extraction conditions ranged over numerous values. At 13.8 MPa extraction pressure, the protein% ranged from 22 to 42%, whereas at 34.5 MPa from 40 to 100°C, it ranged from 26 to 39. These values, and the range of values associated with extraction at 69 MPa and 40–100°C (29–66%) partially reflect the loss of oil from the matrix on these limited extraction runs. Phospholipid values for the extracted meals ranged between 1.4–4.4 mg/g-meal.

Protein and phospholipid meal content as a function of extraction time or volume of CO_2 passage showed no discernible trend. The phospholipid content ranged from 2.7 to 6.1 mg/g-meal for the passage of 100–600 l of CO_2. However, the protein content in this extraction remained high, ranging from 56.0 to 65.9% over the same CO_2 volume interval as noted previ-

ously. Addition of ethanol cosolvent at the three previously specified levels of 51.7 MPa/80°C/200 ml-lCO_2 and 69 MPa/100°C/250 ml-lCO_2 gave very low levels of TFFA in the vernonia meal which ranged from 0.126 to 0.286 mg/g-meal. Protein levels for these two sets of extraction conditions were from 44 to 57%. Interestingly, the phospholipid content of the extracted oil obtained under these cosolvent extraction conditions was higher than those found in the residual meal, and increased to 13 mg/g-oil as the pressure, temperature, and volume of CO_2 used increased, which is consistent with previously reported trends (Montanari et al., 1999).

4. Conclusions

Extraction of oil from *Vernonia galamensis* using SC-CO_2 is possible by optimizing the extraction conditions. Exhaustive extraction of the oil is favored by using high pressures and temperatures, which also minimizes the production of free fatty acids in both the oil and meal by deactivating the lipase enzyme responsible for their production. Total extraction is also aided by application of a two-stage extraction process when using neat SC-CO_2 or the application of a cosolvent, such as ethanol. Furthermore, the free fatty acid content of seed oils can be reduced by supercritical fractionation techniques, through density-based fractionation with SC-CO_2 (Turkay et al., 1996) or a packed column, temperature gradient approach (Dunford and King, 2000). Thus, a two-stage deacidification process for obtaining a fatty acid-free vernonia oil is possible employing SC-CO_2.

Acknowledgements

Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

References

Aldercreutz, P., Gitlesen, T., Ncube, I., Read, J.S., 1997. *Vernonia* lipase: a plant lipase with strong fatty acid selectivity. In: Rubin, B., Dennis, E.A. (Eds.), Lipases, Part A Biotechnology, Methods in Enzymology, vol. 284. Academic Press, San Diego, CA, pp. 220–232.

Anderson, M.A., Collier, L., Dilliplane, R., Ayorinde, F.O., 1993. Mass spectrometric characterization of *Vernonia galamensis* oil. J. Am. Oil Chem. Soc. 70, 905–908.

Ayorinde, F.O., Carlson, K.D., Pavlik, R.P., McVerty, J., 1990. Pilot plant extraction of oil from *Vernonia galamensis* seed. J. Am. Oil Chem. Soc. 67, 512–518.

Ayorinde, F.O., Nwaonicha, C.P., Parchment, V.N., Bryant, K.A., Hassan, M., Clayton, M.T., 1993. Enzymatic synthesis and spectroscopic characterization of 1,3-divernoloyl glycerol from *Vernonia glamensis* seed oil. J. Am. Oil Chem. Soc. 70, 129–132.

Borch-Jensen, C., Mollerup, J., 1996. Determination of vernolic acid content in the oil of *Euphorbia lagascae* by gas and supercritical fluid chromatography. J. Am. Oil Chem. Soc. 73, 1161–1164.

Carlson, K.D., Schneider, W.J., Chang, S.P., Princen, L.D., 1981. *Vernonia galamensis* seed oil: a new source for epoxy coatings. In: Pryde, E.H., Princen, L.H., Mukherjee, F. (Eds.), New Sources of Fats and Oils. Am. Oil Chem. Soc. Monogr. 9, pp. 297–318.

Dunford N.T., King J.W. 2000, Supercritical carbon dioxide fractionation of crude rice bran oil using a packed column with characterization of the resultant fractions, Proceedings of the 5th International Symposium on Supercritical Fluids, 8–12, April 2000, Atlanta, GA, p. 1–10.

Favati, F., King, J.W., Mazzanti, M., 1991. Supercritical fluid extraction of evening primrose oil. J. Am. Oil. Chem. Soc. 68, 422–427.

Favati, F., King, J.W., List, G.R., 1996. In: King, J.W., List, G.R. (Eds.), Effect of supercritical fluids on residual meals and protein functionality. AOCS Press, Champaign, IL, pp. 267–287.

Friedrich, J.P., 1984. Supercritical CO_2 extraction of lipids from lipid-containing materials, US Patent 4,466,923.

Issacs, N.S. (Ed.), 1998. High Pressure Food Science, Bioscience and Chemistry. Royal Society of Chemistry, Cambridge.

King, J.W., 1997. Critical fluids for oil extraction. In: Wan, P.J., Wakelyn, P.J. (Eds.), Technology and Solvents for Extracting Oilseeds and Nonpetroleum Oils. AOCS Press, Champaign, IL, pp. 283–310.

King, J.W., List, G.R. (Eds.), 1996. Supercritical Fluid Technology in Oil and Lipid Chemistry. AOCS Press, Champaign, IL.

List, G.R., Friedrich, J.P., King, J.W., 1989. Supercritical CO_2 extraction and processing of oil seeds. Oil Mill Gazetter 95 (6), 28–34.

Mohamed, A., Bhradwaj, H.L., Hamama, A., Webber, C. III, 1995. Chemical composition of kenaf (*Hibiscus cannabinus* L.). Ind. Crops Prod. 4, 157–165.

Montanari, L., Fantozzi, P., Snyder, J.M., King, J.W., 1999. Selective extraction of phospholipids with supercritical carbon dioxide and ethanol. J. Supercrit. Fluids 14, 87–93.

Neff, W.E., Adlof, R.O., Konishi, H., Weislander, D., 1993. High-performance liquid chromatography of the triglycerols of *Vernonia galamensis* and *Crepis alpina* seed oils. J. Am. Oil Chem. Soc. 70, 449–455.

Nixon, M., Chan, H.P., 1979. A simple and sensitive colorimetric method for the determination of long-chain fatty acids in subcellular organelles. Anal. Biochem. 97, 403–409.

Turkay, S., Burford, M.D., Kemal Sangun, K., Ekinici, E., Bartle, K.D., Cliford, A.A., 1996. Deacidification of black cumin seed oil by selective supercritical carbon dioxide extraction. J. Am. Oil Chem. Soc. 73, 1265–1269.